

QUANTITATIVE DETERMINATION OF VITAMIN
B IN SPINACH AND STRING BEANS, WITH
A PRELIMINARY REPORT ON THE
VITAMIN A CONTENT OF
THE SAME FOODS

by

E. CHARLOTTE ROGERS, B.A., A.M.

*Submitted in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy*

in the

GRADUATE SCHOOL

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TABLE OF CONTENTS

	Page
Introduction.....	5
Aim.....	5
Experimental Methods.....	6
a. Animals.....	6
b. Basal Diet.....	7
c. Vitamin Food.....	7
d. Dry Weight Equivalents.....	8
Experimental Results.....	8
a. Yeast.....	8
b. Spinach.....	8
1. Raw Spinach.....	8
2. Open Kettle Cooked Spinach.....	8
(1). Fifteen-minute Cooked.....	8
(2). Seven-minute Cooked.....	9
3. Canned Spinach.....	9
(1). Home Canned.....	9
(2). Commercial Canned.....	9
c. Beans.....	9
1. Raw String Beans.....	9
2. Open Kettle Beans.....	10
(1). Fifteen-minute Cooked.....	10
3. Home Canned.....	10
4. Commercial Canned.....	10
d. Experiment with Supplements.....	10
1. (1). Whole Wheat.....	11
(2). Beans and Wheat.....	11
2. Whole Wheat and Beans.....	11
3. Corn Concentrate.....	11
4. Potency of Corn Concentrate.....	12
Analysis of Results.....	12
a. Yeast.....	12
b. Spinach.....	12
c. Beans.....	13
d. Supplement.....	13
e. Relation of Weight of Animal to Weight of Vitamin Food.....	14
1. Raw Beans.....	14
2. Open Kettle Beans.....	15
f. Comparison of Winter (Storage) and Fall Beans.....	15
g. Hydrogen ion.....	16
h. Effect of Change of Basal Diets.....	17
Summary of Work on Water-Soluble Vitamins.....	17
Conclusions.....	18
Tables.....	19
Charts.....	29
Preliminary Report on Vitamin A in Spinach and Beans.....	33
Tables.....	35
Bibliography.....	38



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INTRODUCTION

The general vitamin content of many fruits and vegetables has long been known, but the exact quantity of vitamin units are known for a smaller number. Especially does this seem to be true of vitamin B. The difficulties attending the determination of the unit of B in canned peaches and strawberries have been noted by Kohman and Eddy.^{1,2} Burton³ in her study of collard and turnip greens made no attempt to determine the two factors of B, although she considered it probable that the proportion of the antineuritic to the growth factor might have a greater effect at a maintenance than at a higher level.

In attempting a comparative study of the B content of some raw and cooked vegetables, spinach and green beans were chosen because one was typical of the thin leafy vegetables, and the other of the so-called non-acid fruits in which both seed and pericarp are edible, and because it was hoped that they might be obtained all through the year in the local markets. Although some work has been done on both raw and cooked spinach, much of it has been on the dried product⁴, and the aim in the present investigation has been to determine the vitamin B content of spinach and beans in some of the forms in which they are eaten by people.

AIM

The investigation as originally planned was to determine the quantity of vitamins in raw, open kettle cooked, home and commercial canned spinach and string beans. Following an historical sequence, it was decided to begin with vitamin B and most of the work has been done in this group of water soluble vitamins. The report on A may be considered preliminary.

Fresh raw spinach and green string beans were made the basis of comparison for the B content of the cooked and canned products, and at the same time a comparison of the B content of these with the B content of yeast was also attempted, since yeast had long been considered especially rich in vitamin B. The raw spinach and beans and yeast would, therefore, serve as positive controls. The preliminary work November, 1926, to January, 1927, made it seem probable that yeast (Brewer's Yeast-Harris) was not as good a substance for such a comparison as it had been expected to be, and led to a belief that yeast was very rich in the growth factor, but not correspondingly rich in the antineuritic factor. The later work on yeast was merely an attempt to prove this point which meantime was being demonstrated by other workers^{5, 6, 7, 8, 9, 10}. We therefore came to realize early in our experiment that the so called water soluble B vitamin was dual in nature as the work of earlier investigators had sometimes indicated and confirmed the work of Emmett and Luros¹¹. However, it seemed best to continue to determine the quantity of B on the basis of Sherman's growth unit¹², even though it was considered probable that the two factors in perfect combination might be different in their effect on growth as well as on health, from the effect produced by a substance deficient in the antineuritic factor.

The difference between the growth effects and the antineuritic effects of both spinach and beans soon became very evident. Both appeared rich in the growth factor, and both appeared to be somewhat lacking in the antineuritic factor. On this account units of B in accordance with the growth definition given above were not usually obtained. It has therefore been assumed that the unit of maintenance lies

below the smallest weight of the vitamin food per day that enabled animals of 30 to 70 grams weight at 28 to 29 days of age to live through the experimental period. Often they were paralyzed and miserable looking animals that had shown subnormal growth. For example a "yeast" rat, one given 0.2 gram yeast per day was 68 per cent above maintenance at the end of the experimental period and appeared badly paralyzed. On a daily allowance smaller than 0.2 gram, the animals did not live through the period.

Similarly animals on raw beans weighing 35 to 40 grams at 28 to 29 days of age and receiving 2.75 grams of raw beans daily, showed an average gain of 36 per cent combined with 71 per cent of beri-beri. Even in cases where 100 per cent were paralyzed, the growth was 20 per cent or more.

In the case of commercial canned spinach, it seemed well nigh impossible to obtain anything like a unit of B, because the antineuritic factor was so exceedingly small that the animals died of beri-beri, even when they had made good growth gains at first.

As Eddy has stated¹³ it seems necessary to devise new methods of testing for the B vitamins. However, there may still be some value in the study of the combination of growth and antineuritic factors as they occur in foods. So in connection with this work it may be stated that the smallest quantity of spinach or of beans that enabled a rat to live through the experimental period was considered the unit for growth. This was usually coincident with beri-beri so that another unit for health was stated in most cases. An animal was considered to have beri-beri when it was (1) paralyzed, a condition that lasted 10 to 14 days before the animal died; (2) when it was humped and extremely nervous coincident with loss in weight, if at autopsy the animal showed intestines lacking in tone, congested liver and sometimes lungs, dark and sometimes apparently enlarged suprarenals. Often the intestines were gas filled or showed stasis, and usually a bad odor was observed even when the animal was autopsied immediately after death. A in drop weight seemed to accompany both the paralytic and nervous types of beri-beri, so that if the animal had neuritis at the end of the experimental period, a final gain of 20 to 30 per cent rarely represented the maximum growth attained. Frequently an animal with beri-beri died before it had nearly approached the weight at the beginning of the experimental period, and autopsy often showed a pneumonia complication. Other more resistant animals reached the fifty-sixth day of experiment below original weight. Such animals were usually paralyzed with beri-beri or were showing the rapid drop in weight that precedes the paralytic stage of the disease. An attempt was made to find out the value of the growth factor when the antineuritic requirement was met by some substance relatively rich in this material. This work was suggested by the investigation of Sherman and Axtmeyer¹⁰ and of Goldberger⁵.

Throughout this study the effect of cooking or of treatment of the beans and spinach was considered with reference to the destruction of one or both of the B factors.

EXPERIMENTAL METHODS

a. Animals. The experimental animals were albino rats of known inheritance and dietary history. Most of the animals came within the range of 30 to 60 grams. (See tables B1 to B10 for averages of initial weights.) The stock animals, except those used the first three months, were all raised on Sherman's diet 13—two-third whole wheat, one-third klim and salt equal to 2 per cent of the wheat. Following the technique of Sherman and Spohn¹⁴, animals were raised on wire mesh cages, and at 28 or 29 days of age were transferred to smaller cages provided with water bottles

and with raised screen bottoms of 1-4 to 3-8 inch mesh to prevent access to feces. Cages were cleaned six days a week, and as far as possible all basal diet was recovered, and records of food consumption were kept.

b. Basal Diet. The basal diet used in the first part of this experiment, November, 1926, to June, 1927, was a modified Steenbock¹⁵, casein 18, agar 2, Osborn and Mendel salt 4, cod liver oil (Harris medicinal) 1.5, and Argo corn starch to make 100 per cent. After May 26, 1927, the Osborn and Mendel "Standard Food"¹⁶, starch 54, casein 18, butterfat 9, lard 15, and Osborn and Mendel salt 4, was used. Commercial casein for both these diets was purified by extraction with 60 per cent alcohol according to the method described by Sherman and Spohn.¹⁴

c. Vitamin Food. Cod liver oil was fed separate from the rest of the basal diet, as was the vitamin food. The latter was fed seven days a week November, 1926, to February, 1927, and six days a week February, 1927, to May, 1928. All calculations were made on a basis of six feedings a week. That is 0.85 given as a subnormal growth unit of raw spinach means 0.85 gram fed six times a week. The spinach was the best that could be obtained in the local markets. Failure to get string beans in December, 1926, led to abandoning the experiments with beans until the spring of 1927 when the markets had plenty of green southern beans. From mid June to August, string beans from nearby gardens were used. In the late fall of 1927, local market beans became poorer and it was necessary to buy in larger lots and resort to refrigeration. From January to April, 1928, the beans were shipped in twice a week and were fresh, young and tender southern beans.

One lot of spinach was cooked seven minutes and the other was cooked fifteen minutes. The beans were cooked fifteen minutes in order that they might be comparable as regards B loss with spinach cooked the same period of time. In preparing the spinach for use, all root portions or large coarse stems were discarded. the plants were thoroughly washed, and leaves that were to be fed raw were quickly dried on paper toweling before the portions were weighed out. For the open kettle cooked spinach experiments, the washed plants were placed in well covered pans with no water except the small amount that clung to the leaves. The first intention was to cook both beans and spinach just fifteen minutes, but later an experiment on spinach cooked seven minutes was added because some people like it cooked seven to ten minutes much better than fifteen minutes, and it seemed desirable to compare these in vitamin content.

The pans used from November, 1926, to July, 1927, for cooking beans and spinach were aluminum. After July, 1927, that is after the hydrogen ion determinations were made, porcelain lined pans were used.

The best spinach that could be obtained was used for the home canned spinach test, and it was prepared according to directions given in Farmers' Bulletin Number 1476. That is, it was wilted quickly and placed in half pint glass jars and processed 90 minutes at 10 pounds pressure. Two or three cans of spinach were opened each week and the opened cans were kept in a refrigerator when not in use.

For the testing of the B content of commercial canned spinach, Libby's California Fancy Special (15 ounce cans) was chosen because it is a much used and popular brand.

Raw beans were cleaned and freed from strings before portions were weighed out, and for the open kettle cooking they were placed in a small quantity of water and cooked just fifteen minutes. That is the beans remained in boiling water fifteen minutes.

The string beans that were canned were processed according to the directions in Farmers' Bulletin 1471: that is, they were blanched or precooked three minutes

in boiling water, transferred to half-pint glass jars, and processed 40 minutes at 10 pounds pressure.

For the experiment with commercial canned beans, Snider's Cut Refuge Beans (10½ ounce cans) were chosen.

Corn concentrate used in the third experiment with supplements was made by extracting whole ground corn with 85 per cent alcohol, according to Goldberger's method.⁵

d. Dry weight equivalents. The dry weight determinations (See table B18) were made by the usual method of obtaining moisture content, that is drying at a temperature of 98° to 104° centigrade in an electric oven until constant weight was obtained. Spinach and beans that were used during February, 1928, and that seemed to be the same variety as those that were fed throughout the experimental period, were taken in determining the dry weights of the products fed. The home canned spinach was a midsummer canning, and the home canned beans were partly a summer canning and partly a January, 1928, canning of southern beans. The commercial canned spinach and beans were purchased in the spring of 1927 and the winter of 1928.

In weighing out these samples, care was taken to do it exactly as it was done every day when portions were weighed out for the animals.

EXPERIMENTAL RESULTS

a. Yeast. During the late fall of 1926 seven animals were fed daily portions of yeast, varying from 0.1 to 0.3 gram. They grew very rapidly at first, then declined somewhat in weight and developed beri-beri or died of pneumonia. Because of supplementary feeding of tomato juice for two or three days, these animals are not included in the table B1. From May to November, 1927, twelve animals were fed amounts of yeast varying from 0.2 gram to 0.5 gram per day. One on 0.25 gram died and one on the same amount developed beri-beri and two of the three fed 0.2 gram showed marked beri-beri, although the per cent of gain in weight in these groups was 87 and 58 respectively. Those on higher levels, 0.3 gram to 0.5 gram daily, were free from beri-beri and showed gains of 14 per cent to 227 per cent. It would therefore appear that the unit for maintenance on the brewers' yeast-Harris was below 0.2 gram, but the deficiency in the antineuritic factor made it difficult to determine. For example, animal 249 ♂ fed 0.2 gram daily was 64 per cent above maintenance at the end of the eight-week period, but was so paralyzed with beri-beri that he could not have lived much longer.

b. Spinach.

1. Raw spinach. The amount of raw spinach fed varied from 0.96 to 0.75 grams. All told 21 animals were used, but usually only one or two at a time, because a positive control was desired whenever cooked spinach was being fed. Contrary to Eddy's⁴ experience, we find that spinach is eaten raw.

No rat on any quantity above 0.85 gram per day developed beri-beri, but one out of a group of three on 0.85 gram per day developed a temporary paralysis for a week only, and one died of heart disease. The survivors on 0.85 gram averaged 23 per cent above maintenance. The unit of maintenance may lie between 0.8 and 0.85 gram for a 40 to 50 gram rat.

2. Open kettle cooked spinach.

(1). Fifteen-minute cooked. 23 animals received daily portions of 1.6 to 3.5 grams of spinach cooked fifteen minutes. (See table B3). No animal developed beri-beri, and the average per cent gain for a 55 to 60 gram rat on 2 grams per day was 24.3. Two animals on less than 1.5 grams died, but two on 1.6 grams not

only lived but showed an average of 77 per cent gain in weight. It is therefore probable that the unit of maintenance on fifteen-minute open kettle cooked spinach is below 1.6 for a 35 to 40 gram rat or is 1.8 for a 50 to 60 gram rat.

(2). Seven-minute cooked. Amounts between one and two grams of spinach cooked seven minutes were fed to eleven animals. One animal which received daily one gram of spinach developed a severe case of beri-beri and died on the last day of the experimental period 14 per cent above maintenance in weight. Presumably the unit is between 1 and 1.1 for a 35 to 40 gram rat, or between 1.3 and 1.4 for a 40 to 50 gram animal.

3. Canned spinach. Canned spinach as ordinarily served is moist, but not all of the liquid in the can, at least not in the case of a very watery product like the commercial canned type used in this experiment, is eaten. In weighing out the daily portions of canned spinach, there was usually some small excess of liquid left in the can, or was in the few cases where the half-pint can contained a small enough amount for it to be used in two or three days. In no case was all of a number 1, 15 ounce can of the commercial canned spinach used. The home canned spinach was used within three to four months from the time it was canned.

(1). Home canned. Of twenty-four animals used for this experiment, fourteen died and two had beri-beri. (See B5). The large mortality made it difficult to decide on the unit nearest to maintenance, but two rats averaging 50 grams in weight and fed daily 4 grams of this food lived through the eight-week period 18 per cent above maintenance, so it was considered that this amount was near the unit sought. It is however noted that two larger rats on larger amounts, 5 and 5.25 grams, had beri-beri very slightly.

(2). Commercial canned spinach. Thirty animals were used on this experiment, twenty-four died, and all but two of the survivors had beri-beri. One animal with the high initial weight of 69 grams maintained on 9 grams and a 50 gram rat was 11 per cent below maintenance on 6 grams daily. One 63 gram rat 9 per cent more than maintained on 7 grams but two animals, one of which had beri-beri, died on 8 grams. Animal 138 ♀ (receiving 4.5 grams) was badly paralyzed the sixth and seventh weeks, then recovered and lived to the end of the experimental period 23 per cent below maintenance.

The death of 7 out of 8 animals on 5 grams of this spinach suggests this was a case of spontaneous recovery.

Of the above mentioned eight, the seven that died had beri-beri and the eighth came through the experimental period 28 per cent below maintenance and was doubtless in the first stages of beri-beri.

The home canned spinach rats were prone to die and were a poor looking lot, but those on commercial canned spinach were by far the worst and most miserable looking specimens among all the experimental animals. They evidently did not like this food, but all learned to eat it after a few days, and up to the day of death ate usually all that was presented. It seems probable that the unit for maintenance is 6 to 7 grams for a 50 to 60 gram rat, and perhaps 6 grams may be enough for a 40 to 50 gram rat, although the one within that range that received 6 grams of this food developed severe beri-beri and died at the end of the seventh week.

c. Beans.

1 Raw string beans. Fifty-four of the sixty-one animals used for this experiment on raw beans are included in the table B7. Seven animals started on raw beans died before the end of the period or were fed canned beans and are not included in the table. The occurrence of paralysis or of beri-beri in this group was interesting. Five animals fed 3.5 to 4 grams of raw beans showed no sign of this

disease and only two of the sixteen that received 3.25 grams per day were paralyzed. These two belonged to a group that received the early winter or refrigerated beans previously mentioned.

The percentage of beri-beri on 3 grams of raw beans a day was much higher, and on 2.75 grams per day it was as high as 72. It will be noted that the group of eleven in which the incidence of beri-beri was so high were 36 per cent above maintenance at the end of the experiment, and that the average weight was less than that of the group of eighteen that received 3 grams per day. The consideration of a relationship of weight to vitamin requirement will be mentioned later.

From the figures given in table B7, it might be considered that 2.6 to 2.75 grams of raw beans per day represent a unit of B—at least for a rat having an initial weight of 35 to 45 grams. The largest rat in this group, 309 ♂, weighed 47 grams at the beginning of the experimental period and 47 at the end, and had shown a maximum weight of 63 grams, but he was badly paralyzed during the last eight days.

2. Open kettle beans.

(1). Fifteen-minute cooked beans. Open kettle beans in portions varying from 3.5 to 6 grams were fed to 49 animals. None of those fed 4.5 or more grams and only 18 per cent of those receiving 4.25 grams showed any signs of beri-beri, but 58 per cent of the eleven that survived on 4 grams had the disease. An apparent but probably not real discrepancy appeared in the group fed 3.75 grams. Only 28 per cent had beri-beri, but the average initial weight was lower than that of the group on 4 grams. (See B8). Three were fed 3.5 grams. These were paralyzed but averaged 48 per cent above maintenance at the end of the experimental period. In this group was one small (32 gram) rat that was 72 per cent above maintenance, so the average gain is relatively high.

It therefore appears that the unit of maintenance for open kettle beans probably lies between 3.5 and 4 grams for a 40 to 50 gram rat.

3. Home canned beans. Twenty-one animals were used in this experiment. (See B 9). Five animals received 5.5 to 6 grams of home canned beans and throughout the period were free from beri-beri. Two of the three that survived on 5 grams, all four of those on 4.75 grams, and the four on 4.5 grams had the disease. The only one on 4 grams died early in the experiment, presumably from lack of vitamin. It seems probable that the unit for maintenance is between 4.25 and 4.75 grams for a 40 to 50 gram rat.

4. Commercial canned beans. Eleven animals were used in this experiment. The four on 5.5 to 6 grams showed gains of 23 to 36 per cent and were in healthy condition at the end of the experiment. (See B10). One of the two survivors on 5 grams had beri-beri and the one on 4.5 grams not only had beri-beri but survived the test period 12.5 per cent below maintenance. The one that survived and maintained on 4 grams had beri-beri moderately. She had an initial weight of only 30 grams. It would appear from such data as we have that the unit of maintenance might be 4.75 to 5 grams for a 40 to 50 gram rat, but that 5.25 to 5.3 grams are needed for good health.

d. Experiments with Supplements.

1. Experiments with (1) whole wheat and (2) beans and wheat.

It is evident that beans whether raw or cooked are lacking in the antineuritic factor or that the two factors are present in such proportions that it is not easy to determine a unit of maintenance. We found that as we reduced the quantity of vitamin food, approaching the unit of maintenance, the amount of neuritis and the number of deaths from beri-beri increased. Sherman and Axtmeyer showed that milk and wheat were supplementary, the former being rich in G and the later in F. (Following

their terminology we may in the future refer to the antineuritic factor as F and the other factor that combined with F stimulates growth as G.)

It may be that the addition of a small amount of a substance rich in F would greatly lower the amount of spinach and beans and yeast needed for maintenance and so change and clarify our definition of the unit. With this in mind, experiments on wheat, combinations of beans with wheat, also beans with a corn concentrate were undertaken. This opened up a line of work which should repay further investigation.

Four animals that had lived through the experimental period of eight weeks, but were very sick, that is badly paralyzed with beri-beri, were given daily 0.8 grams of whole wheat in place of their former vitamin food, and in one week they were entirely cured and had gained slightly in weight. For eight weeks they maintained weight on this supplement to their B free diet. They were active and free from beri-beri, but by the end of another six to seven weeks they began to show some of the symptoms described by Goldberger and others⁵ as similar to pellagra. Especially interesting were the red scaly areas on the inner edge of the fore feet and thin haired areas over the eyes.

The tenth week of this diet they began to decline in weight and after twenty-one more days or after 85 days on whole wheat, the diet of two of these, 359 ♂ and 361 ♀ was again changed to half the quantity of wheat, 0.4 grams and 2.5 of home canned beans, half the quantity that they had been given in the first experimental period. (See Chart I). Both gained in weight but 359 ♂ became paralyzed (Chart I, 3) so after eight days the diet was changed again to 4.5 grams of beans for 359 ♂ 3.5 grams of beans for 361 ♀ and 0.6 gram whole wheat for each. (Chart I, 4). They began to gain immediately, 359 ♂ was cured of paralysis in four or five days. By the eleventh day 361 ♀ had begun to decline in weight, but appeared well. These animals were now 187 days old, so could hardly be compared with younger experimental animals. It was however evident that 0.4 gram of wheat was not quite enough to cover the antineuritic deficiency of 2.5 grams of home canned beans for animals 6 months old, although there was a slight initial growth stimulus when 2.5 grams beans supplemented 0.4 gram wheat. The addition of 0.6 gram of wheat to 4.5 grams of beans was sufficient to prevent beri-beri and allow for subnormal growth for four weeks or until the experiment terminated.

2. Whole wheat and beans. A group of three animals was given 0.4 gram of whole wheat daily and a small quantity of home canned beans. One rat, 435 ♂ weighing 53 grams on the 29th day was given 2.4 grams of beans and 0.4 gram of wheat. A smaller animal, 442 ♂, weighing 29 grams, was given 2.0 grams of beans with the wheat supplement and a third animal, 437 ♀, that weighed only 18 grams on the 29th day, which is considered too small for usual vitamin studies was given 2.0 grams of commercial canned beans and the 0.4 gram of wheat. All animals gained weight as is shown in Chart II. One of these animals, 442 ♂, was paralyzed for nine days but was 38 per cent above maintenance at the end of the usual eight weeks experimental period. The other animal, 435 ♂ was 45 per cent above maintenance and never showed signs of beri-beri. It therefore appeared that 0.4 gram of whole wheat is barely sufficient to cover the antineuritic deficiency of 2.4 grams of home canned beans.

3. Corn concentrate. Corn concentrate was used to supplement raw beans in a third group of three animals. Two received 3.0 grams and one received 2.75 grams of raw beans and this was supplemented by corn concentrate at levels of 2.5, 1.0 and 0.5 per cent of their basal diet. (See Chart III). Another group of three animals was given commercial canned beans, two receiving 4.75 and one 4.5

grams. This was supplemented by corn concentrate at 2.5, 1.0, and 0.5 per cent levels as in the third group.

In both cases the quantities of beans were chosen as portions which might be expected to have the same antineuritic effect and to be insufficient to prevent beri-beri in animals of their initial weight.

One animal, 447 ♂, died of pneumonia soon after the beginning of the experiments, and a second, 449 ♀, died the 41st day after showing some beri-beri symptoms for a week and real paralysis for four days. The other four lived through the experimental period, but the condition of 445 ♂ was the least satisfactory. This animal, 445 ♂, and the two that died, 447 ♂ and 449 ♀ all received commercial canned beans. All animals on raw beans grew well and showed no signs of beri-beri except for the slight temporary sickness of the one that received 0.5 per cent corn concentrate.

4. Potency of corn concentrate. To test the potency of the corn concentrate curative tests were made on three animals that were paralyzed at the end of the experimental period (See Chart IV). Animal 438 ♂, which was badly paralyzed, was given corn concentrate as the sole source of the B vitamin, at the level of 25 per cent of the basal diet, and appeared entirely cured in two days. The paralysis did not return when on the following days the amount was reduced to 20, 15 and 10 per cent. Two other animals, 441 ♂ and 442 ♀ received at end of the experimental period, corn concentrate, at the level of 5 per cent of the basal diet, in addition to their usual portion of beans. These animals also appeared free from beri-beri in two or three days.

ANALYSES OF RESULTS

a. Yeast. Yeast did not prove to be an especially valuable substance as a positive control, because it was so very rich in the growth factor and relatively low in the antineuritic factor. The incidence of beri-beri was high on a quantity that gave relatively large growth gains, proving that the ratio between the two factors was not optimum for the health of the animals.

b. Spinach. Eddy and Kohman⁴ stated that the B content of spinach was a third to a sixth as great as yeast and that canning did not affect the B content except to the extent of making the rat persistently refuse to eat an adequate supply. If, as they state, 0.3 gram is the daily requirement of yeast for a rat it surely appears that our fresh raw spinach is scarcely more than a third as potent, since our unit was 0.85 gram. Certainly the large mortality of our animals on canned spinach could not be attributed to a refusal to eat it, although we grant that they appeared to dislike this food and to relish very much both raw and open kettle cooked spinach.

Our experiment on spinach covered two years, and during all seasons there seemed to be little difference in the B content of our raw spinach. There is, of course, considerable variation in the water content of spinach. This is shown by a comparison of the analysis given in Bulletin Number 28 and our analysis. (See table B18). If we can assume that the dried raw spinach used by Kohman and Eddy⁴ and by Osborn and Mendel¹⁷ contained even 75 to 80 per cent as much vitamin B before it was dried as our raw spinach, there appears to be a large loss of B content in drying.

The dry weight equivalent of 0.85 gram of our raw spinach is 0.105 gram. According to the analysis of raw spinach given by Kohman and Eddy, the product contained 9.84 per cent solids or 20 per cent less than ours. Osborn and Mendel found that one gram of dried raw spinach was the minimal protective dose. On the basis of the analyses of raw spinach given by bulletin 28 and by Kohman and Eddy, as well as our analysis, there would appear to be an 85 to 90 per cent loss of B in the drying process. In the cooking there seems to be a progressive loss depending upon the temperature and time. A calculation of dry weight equivalents of the units

for maintenance or of subnormal growth indicates a loss of 38 to 41 per cent in cooking spinach 7 minutes, of 46 to 51 per cent in cooking it 15 minutes, of 60 to 63 per cent for home canned and of 78 to 81 per cent for the commercial canned product.

A comparison of the general health of the animals in the different spinach groups, and the high incidence of beri-beri among animals on canned spinach make it evident that there is a progressive destruction of the antineuritic substance in the cooked product which becomes much more marked when the spinach is canned.

That much of the apparent loss of B factors in cooked and canned spinach is due to the destruction of the F factor alone is probable. It may be that it is so completely destroyed or charged that the animal cannot retain its health sufficiently to take advantage of the G factor, or it may be that in the long cooking process both factors are altered.

c. Beans. In the case of raw green beans the deficiency in the antineuritic factor was obvious even in the preliminary group. A comparison of dry weight equivalents of the units chosen for raw, open kettle, home and commercial canned beans gives no suggestion that there is any destruction of vitamin G. However, the higher incidence of beri-beri among the animals that received canned beans and our experiment with corn concentrate gave evidence of some destruction of the F factor in processing.

Since there is no loss in cooked or canned string beans corresponding to the apparent loss in cooked spinach an explanation seems to be demanded. Is the difference due to the great difference in the nature of these two foods? The spinach is a thin leafy substance that wilts and dries easily and the other is a fibrous pod, not easily wilted or dried.

Chamberlain and Vedder¹⁸ said that heating to 120°C a short time destroyed the neuritic-preventing substance, but Sherman and Grose¹⁹ found that only 24 per cent was destroyed by heating at 100°C and 45 to 47 per cent by heating to 120° for four hours. In the light of the present idea that vitamin B has two factors it may be that both statements are correct. There is little doubt that heating affects the antineuritic factor more easily than the growth factor. It may be the arrangement of the vitamin substance is such that destruction is faster in a leafy material like spinach than in a fibrous pod with seeds. Sherman and Grose have suggested that the heat destruction of B "may perhaps be due to the reaction being one which involves two phases of a heterogenous system, the vitamin being in combination with or absorbed upon colloidal material rather than in true solution in the hot water by which it is destroyed." Inasmuch as beans are cooked in water and lose little or none in G content and spinach in its own juice and loses considerable in G content, this theory seems reasonable.

d. Supplements. Our experiment with supplements shows that the quantity of beans necessary for maintenance is lowered or reduced when the antineuritic needs of the animals are fully met by material high in F. The animals that received 2 and 2.4 grams of canned beans supplemented by 0.4 gram of wheat had enough G to allow subnormal growth and the one of them, 435 ♂, receiving 0.4 gram more beans than 442 ♂ was free from neuritis, although the combination, 2.4 grams of beans and 0.4 gram of wheat did not seem to be optimum for excellent physical condition. This animal developed scaly red areas on the fore feet and beaded whiskers and presented an appearance very similar to that shown by 359 ♂ and 361 ♀ after receiving 0.8 gram whole wheat for seven weeks. The condition was, however, not nearly so well developed as it was in the case of the animals that received only whole wheat as sole source of vitamin B.

If the growth stimulating and the antipellagra substances are not one and the

same, it would seem that this animal did not receive the optimum amount of the antipellagra factor in the above mentioned combination of vitamin foods. If the antineuritic needs of the animal are met by a pure antineuritic concentrate, instead of one containing considerable of the G factor, 2 grams of home canned beans which is less than half the quantity previously shown to be the unit for maintenance, may be found to meet the vitamin G needs of the animal.

From the experiment on beans combined with corn concentrate it appears that 0.5 per cent of corn concentrate covered the antineuritic deficiency of 2.75 gram of raw beans. Probably a smaller quantity of raw beans with 0.5 per cent concentrate would have held the animal nearer to a maintenance level since 2.75 grams and 0.5 per cent corn concentrate produced 41 per cent gain in weight. When 4.75 grams of commercial canned beans were fed with 0.5 per cent of corn concentrate, the combination did not prevent beri-beri, and 2.5 per cent was barely adequate. Since 4.75 grams of the commercial canned beans are equivalent to 2.75 grams of the raw in dry weight, and since the former needs 4 to 5 times the corn concentrate required by the latter, this confirmed, previous evidence on incidence of beri-beri (see page 8) and makes it evident that the canning process greatly injures the antineuritic factor. Corn extract, the only antineuritic concentrate available to us at the time of this experiment, probably contains a small quantity of G, so that work with a pure antineuritic concentrate should only confirm these results as to the qualitative destruction of vitamins.

A series of experiments in which some excellent antineuritic concentrate is fed in combination with spinach and beans, and autoclaved yeast in combination with these vegetables, will be needed before we can know the quantitative value of the F and G factors of these foods.

e. Relation of weight of animals to weight of vitamin food. Early in this series of experiments, the difference in the response of animals of different weight at beginning of the experimental period, that is at the age of 28 or 29 days, to a given weight of the vitamin food was noted. Was there really a relation between the initial weight of the animal and the quantity of vitamin required for maintenance and health? Osborn and Mendel²⁰ noted that a definite quantity of yeast would maintain an animal up to a certain weight and that more yeast must be given if weight progressed beyond that point, and Sherman and McArthur²¹ showed that a large animal gained less than a small one on the same weight of vitamin food.

1. Raw beans.

(1). 3.25 gram raw beans. Table B11 shows seven groups of animals on raw beans. There are more animals on this food than on any other, so these groups seemed to offer the best data for such a study of relationship. In group one there were only two animals, but they deviated only 2 grams in weight and averaged 70 grams and showed an average gain of 5 per cent. The slightly heavier one was 14 per cent below maintenance and the other 11.5 per cent above. Inasmuch as the difference in initial weight of these animals was only 2.8 per cent and the difference in maintenance was about 10 per cent, it may be that difference in sex or in individuals (for both belonged to the same litter) accounts for this discrepancy.

The average weight of the four animals in group II was approximately 55 grams and the per cent gain was nearly 25 as compared with 5 above. It may be noted here that the deviations in gain are greater than in the first group, but four different litters are represented instead of one. Seven animals (group III) that showed a maximum deviation of only five grams had an average weight of 43 to 44 grams and showed an average gain of 53 per cent or double that of the group averaging 55 grams in weight.

The two animals of group IV were alike in litter, and initial weight, and showed a difference of four per cent in gain. It is therefore evident that the average gain of a 35 gram rat was 65 per cent, that of a 40 to 45 gram rat was 58 per cent, that of a 55 to 60 gram rat was 25 per cent, and that of a 70 gram rat was 5 per cent on the same daily portion.

(2). 3.00 grams. Table B11 shows also a group of seven that were fed 3 grams of raw beans per day. These animals showed a maximum deviation of 8 grams and averaged about 46 grams in weight. They showed a gain of 34 per cent, that is less than that of the 45 to 50 gram group on the slightly larger amount of vitamin food.

(3). 2.75 grams. A comparison of two groups (VI and VII, table B11) shows the same general relationship, namely a much greater gain for 30 to 36 gram animals than for 40 to 47 gram animals on the same weight of raw beans.

2. Open kettle beans.

(1). 4 grams. Two groups of animals on open kettle beans (table B12) behaved in a very similar way. A group of five that averaged 44 to 45 grams in weight showed a gain of 38 per cent, and a group of three averaging 37 grams showed a gain of 72 per cent when all were fed 4 grams of the beans.

No attempt has been made to determine a mathematical ratio between initial weight and per cent gain, but the general relationship namely the greater the initial weight, the less the per cent gain, was noted in all cases where there were a sufficient number of animals on the same weight of vitamin food.

f. Comparison of winter (storage) and fall beans. It is possible that some of the discrepancies in per cent of gain may be accounted for by differences in beans at different seasons. No particular study of this was intended, so tables B14 and B15 are suggestive only. This series of experiments extended over too long a period of time to make it possible to have beans of uniform size and quality although the best that our markets afforded were used.

It should be noted that in the groups presented to show relation of size to weight of vitamin food (see tables B11 and B12), animals that were fed mostly refrigerated beans were excluded. The comparison presented in B14 is between five animals given 4 grams open kettle beans that were kept in the ice box during a six-weeks period, with ten animals fed on good quality of fresh beans, partly native or local beans. Group I showed an average loss of 7.6 per cent and group II a gain of 45 to 46 per cent, making a total difference of 54 per cent. There was, however, a difference of 18 grams in the average weight of the animals of the two groups, so it might be fairer to take the two animals in group I weighing 61 to 62 grams and the two in group II weighing 62 grams and compare the per cent gains. The two on fall beans showed a gain of about 5 per cent and those on winter beans a loss of approximately 3 per cent or a total difference of 8 per cent. Unfortunately there were no animals in the 35 to 40 gram range, in the early winter group on 4 grams of open kettle beans to make possible a comparison within this range. There were two animals that weighed 71 and 69 grams respectively that showed in the fall experiment an average gain of 5 per cent on 3.25 grams raw beans, and two weighing 81 and 60 grams or averaging like the others that showed a loss of 3 per cent on the same quantity of early winter beans.

Another difference in these string beans was in the size and maturity of the seeds. Probably those fed September 20 to November 11 had the larger seeds, but whether they were more mature is less certain. Eddy and Kohman²² state "that the more mature the pea in the range studied the richer the content with B vitamin." The same may be true of string beans. However, the string beans used November 15

to January 10 were much more mature than were desired, and were by no means as young and crisp as the southern beans used during the remainder of the winter.

g. HYDROGEN ION CONCENTRATION.
P H OF JUICES.

1927	Raw Spinach	Seven-Minute Spinach		Fifteen-Minute Spinach		Home Canned Spinach	Commercial Canned Spinach
		Pan Aluminum	Pan Agate	Pan Aluminum	Pan Agate		
3/26	6.40			6.82		4.85* ¹	5.29* ²
4/9	6.35			7.25		5.88 (4/2)	5.93
7/11	6.80	6.95		7.12		6.45 (6/7)	5.95
7/29						6.10 (7/2)	5.77
7/30		6.85	6.70	6.75	6.65		
Average	6.53	6.90	6.70	5.99	6.65	5.83	5.74

Fifteen-Minute Beans

1927	Raw Beans	Pan Aluminum	Pan Agate	Home Canned	Commercial Canned
3/26					
4/9	6.31	6.32		5.65	5.60
	6.25				
7/11	6.62	7.04		5.75 (7/2)	5.55
7/29	6.35		6.3	5.66 (7/2)	5.45
7/30		6.50	6.40		
			6.45		
Average	6.38	6.62	6.38	5.69	5.53

Note—

*¹ Opened 3 days.

*² Opened 2 days.

(Date) = Dates of canning.

Since Sherman and Burton²³ found that the vitamin content of tomato juice was affected by a change of pH much more than by heating, hydrogen ion determinations were made for the juices of all the kinds of spinach and beans used. The juices were extracted by means of a plant press, and were kept on ice during the one or two hours that sometimes elapsed before the hydrogen ion experiment was begun. A Leeds and Northrup potentiometer and quinhydrone electrodes were used.

The greater acidity of the canned products, and the similarity in pH of raw and open kettle cooked beans and spinach are what might be expected. The close similarity of pH of the home canned and commercial canned food and the very slightly greater acidity of the commercial canned may be of interest. The very slight swing towards alkalinity when spinach and beans were cooked in aluminum pans should not be considered significant until more determinations are made.

Sherman and Burton²³ found that increased alkalinity increased the destruction of B in tomato juice. In the case of spinach and beans the normal change that accompanies long cooking and canning is toward greater acidity which, however, alters the ratio of (H+) to (OH-) in the raw juices, just as addition of alkali to an acid juice like tomato alters this ratio in the other direction.

The vitamin content of open kettle spinach was less affected than that of canned spinach. The pH of the former, at least when cooked in a porcelain lined pan appeared to remain practically the same as the raw food so that vitamin destruction cannot in that case be attributed to changed pH; also, the change from 6.525 pH for raw spinach to 5.735 in commercial canned spinach does not seem large enough to account for the destruction of the F factor.

h. Effect of change of basal diet. Our failure with two groups of animals on string beans, one the preliminary group of November to December, 1926, and the second a group of April to May, 1927, suggested that the appetite and capacity of the animal for the basal diet might be affected by a relatively large quantity of a fibrous material like string beans.

It was decided to change the basal diet, and the lot started May 26 received the Osborn and Mendel fat diet.¹⁶ From this time on the fat diet was used for all animals on string beans and after June for all experimental animals. The fact that relatively fewer animals died after March 26, 1927, than died before that date cannot be entirely accounted for by a change of diet. A change in the floor of the rat room probably reduced the incidence of germ diseases, and our experimental animals doubtless became more uniform as time went on.

Table B13 shows the response of two groups of animals on string beans, raw and cooked, and the amount of basal diet consumed by each animal. The late spring group consumed on an average 4.74 grams per rat per day of the high starch diet, and the fall group (one summer animal included) averaged 3.68 grams of the high fat diet. Immediately it appears that the caloric intake of the two groups is approximately the same or at best there is only about 2 per cent in favor of the high fat diet. This alone may not account for the better results on the fat diet. The fact that some of the animals on the high starch diet, at autopsy showed a somewhat crowded intestine gave an idea that the intestinal condition might be better if the animal did not have to eat so much basal diet. The experiments of Evans and Burr²⁴ with highly purified and with commercial diets suggests the probability of some growth stimulating property in the fat. As opposed to this, two animals used as negative controls might be mentioned. They were litter mates of the same sex and weighed the same amount on the 28th day. One given the high starch diet lived 27 days and the other given the fat diet lived 28 days.

SUMMARY OF WORK ON WATER SOLUBLE VITAMINS

1. Yeast is especially rich in G, but deficient in F, since 0.2 gram daily gives an average gain of 58 per cent with 67 per cent of beri-beri.

2. Raw spinach is rich in the growth factor since 0.8 to 0.85 gram is sufficient for subnormal growth, but it is somewhat deficient in F since a gain of 23 per cent is accompanied by 33 per cent of beri-beri.

3. Open kettle spinach cooked seven minutes appears to be 33 to 41 per cent less potent than the raw product since 1.2 grams gives subnormal growth. This calculation as well as similar ones that follow is based on equivalent dry weight determinations.

4. Similarly the same amount of spinach cooked fifteen minutes that gives the least growth for a 30 to 40 gram rat lies below 1.6 grams and below 2.0 grams for a 60 gram rat or is estimated to be 1.6 to 1.7 grams for a 40 to 50 gram rat. The loss of potency of spinach cooked this way appears to be approximately 45 to 51 per cent.

5. Home canned spinach, processed 90 minutes, appears to have 60 to 63 per cent less effect in maintaining growth and health than raw spinach. Minimum growth was obtained with 4.0 to 4.5 grams.

6. The mortality with commercial canned spinach was 87 to 90 per cent, so it was not easy to determine a "unit", but the survival of one 50 gram animal on 6.0 grams of spinach, one 60 gram animal on 7.0 grams and one 70 gram animal on 9 grams would indicate that 6.0 to 7.0 grams is needed to enable an animal to live. Up to a 9.0 gram level there was so much beri-beri that it is evident that commercial canned spinach is very low in F.

The apparent loss of potency in spinach cooked this way is 78 to 81 per cent.

7. The occurrence of beri-beri to the extent of 72 per cent in a group that received 2.75 grams of raw beans and the fact that they averaged 36 per cent above maintenance indicated that beans are deficient in F. This applies to beans cooked and canned as well as raw. For rats weighing 40 to 50 grams on the 28th day, minimum growth was obtained with 2.75 grams of raw beans.

8. For a 35 to 40 gram rat, 3.5 to 3.75 grams of open kettle beans seems to give the least growth. Beri-beri seemed no more common than when raw beans were used.

9. The amount of home canned beans that gives least growth seemed to be 4.75 grams for a 40 to 50 gram rat or below. There seems to be destruction of F.

10. To allow for subnormal growth of a 40 to 50 gram rat, 4.75 to 5.0 grams of commercial canned beans are needed.

There seems to be destruction of F just as with home canned beans.

11. The above mentioned weights of string beans allowing subnormal growth appear to be the same when dry weight equivalents are used. There is therefore no evidence of loss of G in our beans in the cooking and canning processes but there is loss of F.

12. Whole wheat is rich enough in F, so that 0.8 gram per day will cure in a few days animals badly paralyzed with beri-beri, but this portion does not give enough of G for anything more than maintenance.

13. A slightly higher level of subnormal growth than that obtained in the so-called unit of canned string beans results from the feeding of a combination of 0.4 gram of whole wheat and a half unit of beans.

14. The addition of 0.5 per cent of corn concentrate to a unit for subnormal growth on string beans prevents beri-beri, or allows for subnormal growth at a higher level than that induced by beans alone.

15. It seems probable that a relation exists between the initial (28 to 29 day) weight of the animal and the required amount of vitamin. Briefly the smaller the animal, the less the requirement.

16. It is suggested that storage of string beans affects the F factor.

17. The change in pH in canning spinach and string beans is about 0.8 of a unit toward greater acidity.

CONCLUSIONS

Cooking and canning beans seem to affect the content of water soluble vitamins in spinach, causing a loss of from 38 per cent for seven minute open kettle cooked spinach to approximately 78 per cent for the commercial canned spinach. How much of this loss is due to injury to the F factor cannot be determined until more quantitative determinations of F and G separately have been made.

Cooking and canning does not affect the content of G in string beans. Canning does injure the F content.

It is suggested that a narrower range of initial weights be used in determinations of vitamin B units. The rat which weighs 40 to 55 grams on the 28th or 29th day appears to us most suitable for this purpose.

Quantitative determinations of vitamin B up to the present time measure only the effect of the combined multiple factors and need to be supplemented by quantitative determinations of each factor separately.

TABLE B1
AVERAGES OF ANIMALS ON YEAST

Total Number Animals	Number Died*1	Grams Fed	Average Initial Weight*2 in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
1	0	0.5	66	14.4	0	102♂
1	0	0.4	87	81.6	0	141♂
1	0	0.35	30	226.6	0	172♀
2	0	0.3	44	132.2	0	306♀ 311♂
4	1	0.25	44.5	87.2	33 1/3	56♂ 58♀ 56♀
3	0	0.2	43.1	57.9	66 2/3	281♂ 288♀ 291♂

Note—

During preliminary period, there were 7 rats on yeast. Amounts varying from 0.15 to 0.3 gram. 3 died, rest were supplementary fed with tomato juice to enable recovery from beri-beri.

Note—

Unit for maintenance = below 0.20 gram for 40 to 50 gram rat. Unit for health = 0.25 gram for 40 to 50 gram rat.

*1 Number that died is given because many of these animals died of beri-beri, during the time (1926-1928) of these experiments.

*2 No animal dying before the end of the experimental period is included in any average.

TABLE B2
AVERAGES OF ANIMALS ON RAW SPINACH

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
1	1	1.96	52	44.2	0	
1	0	1.62	47	57.4	0	709R♀
6	4	1.16	55	41.9	0	202R♂ 405R♀
1	0	1.10	70	0.0	0	82♂
3	0	1.00	62	1.4	0	21♂ 103♂ 139♀
2	0	0.90	47	17.6	0	79♀ 130♂
1	0	0.88	33	142.4	0	171♀
3	1	0.85	47	23.2	T	1002R♀ 1209R♂
1	0	0.80	45	62.2	100	426♂
2	2	0.75	--	----	---	

Note—

Two only on Osborn and Mendel diet.

Rest on high starch.

T—1209R♂ had spontaneous recovery after showing signs of beri-beri for 7 days.

Note—

Unit for maintenance = 0.8 to 0.85 gram for 40 to 50 gram rat. Unit for health = 0.88.

See notes *1 and *2, Table B1.

TABLE B3
AVERAGES OF ANIMALS ON FIFTEEN-MINUTE SPINACH

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
1	1	3.50	--	----	--	
2	1	2.62	72	52.7	0	1102♂
1	0	2.25	59	-8.4	0	262 ♀
2	1	2.04	50	42	0	1206 ♀
9	3	2.00	59	24.3	0	132♂ 16 ♀ 17♂ 125♂ 131♂
2	0	1.80	40	79	0	418♂ 419♂
1	1	1.75	--	----	--	
2	0	1.60	37	77.2	0	423♂ 436♂
1	1	1.46	--	----	--	
1	1	1.16	--	----	--	

Note—

Unit for maintenance = below 20 grams for 50 to 60 gram rat, and below 1.6 grams for 35 to 40 gram rat.

Unit for health = below 2 grams.

See notes *1 and *2, Table B1.

TABLE B4
AVERAGES OF ANIMALS ON SEVEN-MINUTE SPINACH

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
1	0	2.00	53	18.9	0	137 ♀
2	0	1.75	49	57.3	0	133♂ 142♂
2	0	1.50	44	40.9	0	143 ♀ 144♂
2	0	1.40	37	58.1	0	420♂ 421♂
2	0	1.20	36	68.0	0	422♂ 424 ♀
2	2	1.00	--	----	-	*

Note—

Unit for maintenance = below 1.2 to 1.1 grams for 35 to 40 gram animal.

Unit for health = presumably 1.3 to 1.4 gram for a 40 to 50 gram animal.

*One of these animals, 144♂, died the 56th day.

See notes *1 and *2, Table B1.

TABLE B5
AVERAGES OF ANIMALS ON HOME-CANNED SPINACH

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
1	0	9.0	60	46.7	0	180 ♀
1	0	8.0	62	45.1	0	179 ♂
1	1	7.0	--	----	--	
2	1	6.0	60	-3.3	0	126 ♂
1	1	5.5	--	----	--	
1	0	5.25	54	13.0	Slight	166 ♂
3	1	5.0	66	-5.1	50 Slight	100 ♂ 50 ♂
4	2	4.5	51	12.5	50	136 ♀ 438 ♂
1	1	4.4	--	----	--	
1	1	4.25	--	----	--	
4	2	4.0	51	18.3	0	77 ♂ 111 ♀
1	1	3.75	--	----	--	
1	1	3.25	--	----	--	
2	2	2.75	--	----	--	

Note—

Unit for maintenance = 4 grams for 40 to 50 gram rat.

Unit for health = 5 to 5.25 grams.

See notes *1 and *2, Table B1.

TABLE B6
AVERAGES OF ANIMALS ON COMMERCIAL CANNED SPINACH

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
1	0	9.0	69	1.4	0	173 ♂
2	2	8.0	64	9.4	50	413 ♂
2	1	7.0	63	26.9	50	178 ♂
3	2	6.0	53	-11.0	*?	127 ♂
3	2	5.5	48	6.3	*?	129 ♂
8	7	5.0	58	-27.5	*?	135 ♀
1*1	0	4.5	49	-22.6	*?	138 ♀
1	1	4.4	--	----	--	
3	3	4.0	--	----	--	
1	1	3.5	--	----	--	
1	1	3.25	--	----	--	
2	2	3.0	--	----	--	
2	2	2.6	--	----	--	
2	2	2.0	--	----	--	

*1 Paralysis for 2 weeks, recovered before end of experiment.

*? Evidences of beri-beri were not found until autopsy.

Note—

Unit for maintenance = 6 to 7 grams for 50 to 60 gram rat; 9 grams for 60 to 70 gram rat.

Unit for health = ?

See notes *1 and *2, Table B1.

TABLE B7
AVERAGES OF ANIMALS ON RAW BEANS

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
2	0	4.0	54	42.7	0	86 ♀ 149 ♂
2	0	3.75	53.5	32.2	0	83 ♂ 150 ♂
1	0	3.5	43.0	76.7	0	151 ♂
18	2	3.25	51.5	39.8	12.5	301 ♂ 320 ♂ 332 ♂ 261 ♂ 276 ♂ 279 ♀ 285 ♂ 279 ♀ 65 ♂ 152 ♂ 161 ♀ 241 ♂ 245 ♀ 247 ♂ 251 ♂
16	4	3.0	47.4	37.7	33.3	239 ♂ 243 ♀ 249 ♂ 253 ♀ 259 ♀ 273 ♂ 283 ♀ 287 ♀ 295 ♂ 307 ♂ 322 ♀ 323 ♀ 446 ♂ 448 ♀
10	3	2.75	38.7	35.9	71.4	274 ♂ 75 ♀ 284 ♀ 294 ♀ 303 ♂ 309 ♂ 313R ♀ 450 ♀
1	1	2.5	----	----	----	
1	1	2.0	----	----	----	

Note—

Unit for maintenance = 2.6 to 2.75 grams for 35 to 45 gram rat; 2.75 to 3.0 grams for 40 to 50 gram rat; 3.0 to 3.25 grams for 45 to 55 gram rat.

Unit for health = 3.25 grams.

See notes *1 and *2, Table B1.

TABLE B8
AVERAGES OF ANIMALS ON OPEN KETTLE BEANS

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
1	0	6.0	54	40.7	0	160 ♀
2	0	5.5	46.5	34.1	0	85 ♂ 159 ♀
1	1	5.25	36	13.9	0	168 ♂
2	1	5.0	42	75.1	0	67 ♂ 155 ♀
1	0	4.75	41	129.3	0	154 ♀
2	0	4.5	50.6	38.9	0	109 ♂ 153 ♀ 118 ♀ 169 ♀ 248 ♂
13	3	4.25	51.18	37.9	19	252 ♂ 256 ♀ 264 ♂ 270 ♂ 277 ♂ 278 ♂
17	6	4.0	47.27	43.8	58	240 ♂ 250 ♂ 254 ♀ 257 ♀ 271 ♂ 280 ♀ 290 ♂ 296 ♂ 308 ♂ 324 ♀

7	0	3.75	38.43	24.4	28	314 ♀	272 ♂	265 ♂
3	0	3.5	38.0	47.8	100	266 ♂	293 ♀	304 ♂
						434 ♀	441 ♂	379 ♀

Note—

Unit for maintenance = below 3.75 grams for a 35 to 40 gram rat, or between 3.5 and 4.0 grams for a 40 to 50 gram rat.

Unit for health = 4.25 grams.

See notes *1 and *2, Table B1.

TABLE B9
AVERAGES OF ANIMALS ON HOME CANNED BEANS

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
2	1	6.0	52	25.	0	162 ♀
3	0	5.5	49.5	16.6	0	117 ♀ 163 ♀ 164 ♀
6	2	5.0	51.3	53.1	50	148 ♀ 155 ♀ 359 ♂
						378 ♂
4	0	4.75	43	17.8	100	360 ♂ 379 ♂ 380 ♂
						381 ♀
5	1	4.5	45.8	22.0	100 severe except smallest	433 ♀ 361 ♀ 382 ♀
						383 ♀ 384 ♀
1	1	4.0	----	----		

Note—

Unit for maintenance = below 4.25 to 4.75 grams for 40 to 50 gram animal.

Unit for health = 5 grams or above.

See notes *1 and *2, Table B1.

TABLE B10
AVERAGES OF ANIMALS ON COMMERCIAL CANNED BEANS

Total Number Animals	Number Died	Grams Fed	Average Initial Weight in grams	Average Per cent Gain	Per cent Beri-beri or Paralysis	Serial Number and Sex
2	0	6.0	51.5	36.3	0	84 ♂ 45 ♂
2	0	5.5	45.0	22.3	0	146 ♀ 165 ♀
4	2	5.0	42.5	15.6	50	147 ♀ 170 ♀
1	0	4.5	40.0	-12.5	100	390 ♀
2	1	4.0	30.0	0	100	391 ♀

Note—

Unit for maintenance = 4.75 to 5 grams for 40 to 50 gram rat.

Unit for health = above 5.25 grams.

See notes *1 and *2, Table B1.

TABLE B11

RELATION OF WEIGHT OF ANIMAL TO WEIGHT OF VITAMIN FOOD (RAW BEANS)

I. 3.25 grams. 60 to 70 grams.

Date	Number Days	Serial No. and Sex	Vitamin Food and Amount	Initial Weight	Per cent Gain
9/24-11/19	56	279 ♀	Raw Beans 3.25	69	11.6
9/24-11/19	56	276 ♂	Raw Beans 3.25	71	-1.4
			Average	70	5.1
	II.	3.25 grams.	50 to 60 grams.		
6/1-7/27	56	161 ♂	Raw Beans 3.25	54	40.7
9/20-11/15	56	247 ♂	Raw Beans 3.25	53	52.8
9/30-11/26	56	285 ♂	Raw Beans 3.25	51	0
9/20-11/15	56	332 ♂	Raw Beans 3.25	60	5
			Average	54.5	24.6
	III.	3.25 grams.	45 to 50 grams.		
5/26-7/21	56	150 ♂	Raw Beans 3.25	44	70.7
5/26-7/21	56	151 ♂	Raw Beans 3.25	43	76.3
9/20-11/15	56	251 ♂	Raw Beans 3.25	44	56.8
10/1-11/28	56	297 ♀	Raw Beans 3.25	45	55.5
5/26-7/21	56	152 ♂	Raw Beans 3.25	42	23.8
9/21-11/16	56	261 ♂	Raw Beans 3.25	41	60.9
10/1-11/28	56	301 ♂	Raw Beans 3.25	46	19.6
			Average	43.57	53.37
	IV.	3.25 grams.	30 to 40 grams.		
9/20-11/15	56	241 ♂	Raw Beans 3.25	35	67.1
9/20-11/15	56	245 ♀	Raw Beans 3.25	35	62.9
			Average	35	65.0
	V.	3.00 grams.	40 to 50 grams.		
9/20-11/15	56	239 ♂	Raw Beans 3.00	44	47.7
9/20-11/15	56	243 ♀	Raw Beans 3.00	41	75.6
9/20-11/15	56	249 ♂	Raw Beans 3.00	46	30.4
9/20-11/15	56	253 ♀	Raw Beans 3.00	49	42.3
9/26-11/21	56	283 ♀	Raw Beans 3.00	41	14.6
9/30-11/26	56	287 ♀	Raw Beans 3.00	49	8.2
10/8-12/3	56	307 ♂	Raw Beans 3.00	49	19.1
			Average	45.6	33.7
	VI.	2.75 grams.	40 to 50 grams.		
9/25-11/21	56	284 ♀	Raw Beans 2.75	40	10
10/3-11/30	56	303 ♂	Raw Beans 2.75	43	23.3
10/8-12/3	56	313 ♂	Raw Beans 2.75	47	0
10/8-12/3	56	313 ♀	Raw Beans 2.75	40	4.3
			Average	42.5	9.4
	VII.	2.75 grams.	40 to 50 grams.		
9/21-11/16	56	274 ♂	Raw Beans 2.75	30	83.3
9/21-11/16	56	275 ♀	Raw Beans 2.75	35	91.4
9/30-11/28	56	294 ♀	Raw Beans 2.75	36	38.9
			Average	33.7	71.2

TABLE B12
RELATION OF WEIGHT OF ANIMAL TO WEIGHT OF VITAMIN FOOD
4.00 GRAMS. FED OPEN KETTLE BEANS.

Date	Serial Number and Sex	Initial Weight in grams	Per cent Gain
9/20-11/5	240♂	41	46.3
9/20-11/5	250♂	45	42.2
9/21-11/16	271♂	41	36.6
9/30-11/25	290♂	47	48.8
10/8-12/3	308♂	48	14.6
	Average	44.4	37.7
10/1-11/28	296♂	40	40
9/20-11/15	257♀	35	125.7
9/21-11/15	254♀	37	51.4
	Average	37.3	72.3

Note—

Average per cent gain on 4 grams Open Kettle Beans.

30 to 40 gram rats 72 per cent.

40 to 50 gram rats 38 per cent.

TABLE B13
BEHAVIOR ON HIGH FAT AND STARCH DIETS

Date	Num- ber Days	Serial Num- ber and Sex	Litter Number	Vitamin Food and Amount	Initial Weight in grams	Per cent Gain	Basal Diet in Grams	Grams per Day
HIGH STARCH DIET								
3/26-5/12	56	65♂	G1-2 (G1)	Raw Beans 3.25	51	5.9	264	4.7
3/26-4/24	29	70♂	G1-2 (G1)	Raw Beans 3.25	45	8.44	138	4.7
3/26-5/10	45	66♂	G1-2 (G1)	Raw Beans 3.00	49	8.33	198	4.4
3/26-5/10	45	73♀	G1-2 (G1)	Raw Beans 3.00	44	9.1	199	4.4
3/26-5/6	41	67♂	G1-2 (G1)	Open Kettle 5.00	49	24.5	213	5.2
3/26-5/12	47	68♂	G1-2 (G1)	Open Kettle 4.00	49	16.3	232	4.9
3/26-4/15	20	74♀	G1-2 (G1)	Open Kettle 4.00	39	33.3	120	6.0
3/26-4/22	27	75♀	G1-2 (G1)	Home Canned 5.00	37	10.8	132	4.1
3/26-5/14	49	72♂	G1-2 (G1)	Commercial Canned 5.00	43	4.7	214	4.3
				Average				4.7
HIGH FAT DIET								
5/26-7/21	56	152♂	Outside Litter	Raw Beans 3.25	42	23.8	195	3.5
9/20-11/15	56	241♂	G1-2 (x3)	Raw Beans 3.25	35	62.9	198	3.6
9/20-11/15	56	239♂	G1-2 (x3)	Raw Beans 3.00	44	47.7	228	4.0
9/30-11/26	56	287♀	198 (182)	Raw Beans 3.00	49	8.2	215	3.8
5/26-7/21	56	155♀	Outside Litter	Open Kettle 5.00	35	122.8	234	4.2
9/20-11/15	56	240♂	G1-2 (x3)	Open Kettle 4.00	41	46.3	201	3.6

TABLE B13 (CONTINUED)

Date	Number Days	Serial Number and Sex	Litter Number	Vitamin Food and Amount	Initial Weight in Grams	Per cent Gain	Basal Diet in Grams	Grams per Day
5/26-7/21	56	148 ♀	Outside Litter	Home Canned 5.00	35	65.7	187	3.3
5/26-7/21	56	147 ♀	Outside Litter	Commercial Canned 5.00	41	26.8	195	3.5
					Average			3.7

Note—

G1-2 had excellent litter in spring and fall. In spring experiment, from 3/26 to 5/12, all died but No. 65. On fall experiment, from 9/20 to 11/15, all lived.

TABLE B14
COMPARISON OF ANIMALS ON FALL AND WINTER BEANS
(OPEN KETTLE)

Dates	Number Days	Serial Number and Sex	Litter Number	Grams Fed	Initial Weight in grams	Per cent Gain	Condition
			I. WINTER				
11/15-1/10	56	324 ♀	G21 (63)	4.0	71	-5.7	Beri-beri severe
11/15-12/30	45*	333 ♂	206 (209)	4.0	56	-21.4	Beri-beri severe
11/15-1/3	49*	334 ♀	206 (209)	4.0	61	-18.0	Beri-beri severe
11/18-12/30	42*	335 ♂	x12 (113)	4.0	62	-19.3	Beri-beri severe
11/18-1/13	56	336 ♀	x12 (113)	4.0	53	-9.4	Beri-beri severe
			Average	of survivors	62	-7.6	
			II. FALL				
9/20-11/15	56	240 ♂	G21 (63)	4.0	41	46.3	Excellent
9/20-11/15	56	250 ♂	G21 (63)	4.0	45	42.2	Excellent
9/20-11/15	56	254 ♀	G21 (63)	4.0	37	51.4	Excellent
9/20-11/15	56	257 ♀	95 (94)	4.0	35	125.7	Excellent
9/20-10/1	12*	278 ♂	62 (156)	4.0	34	17.3	Excellent
9/21-11/16	56	271 ♂	x12 (113)	4.0	41	36.6	Excellent
c/24-11/19	56	280R ♀	62 (156)	4.0	62	3.2	Excellent
9/30-11/25	56	290 ♂	217 (182)	4.0	47	48.8	Excellent
10/1-11/28	56	296 ♂	195 (180)	4.0	40	40.0	Excellent
10/1-11/28	56	308 ♂	205 (204)	4.0	48	14.6	Excellent
			Average	of survivors	44	45.5	

*Note—

Higher rate of mortality in winter group than in fall group.

TABLE B15
COMPARISON OF ANIMALS ON FALL AND WINTER BEANS (RAW)

Dates	Number Days	Serial Number and Sex	Litter Num- ber	Grams Fed	Initial Weight in grams	Per cent Gain	Condition
9/24-11/19	56	276♂	62 (156)	3.25	71	-1.408	Excellent
9/24-11/19	56	279♀	62 (156)	3.25	69	11.59	Excellent
				Average	70	5.09	
11/15-1/10	56	320♂	921 (63)	3.25	81	-11.1	Beri-beri severe
11/15-1/10	56	332♂	206 (209)	3.25	60	5	Beri-beri severe
				Average	70.5	-3.05	

TABLE B17
CALCULATED UNIT FOR SUBNORMAL GROWTH* FOR 40 TO 50 GRAM RAT

Food	Grams Moist Food	Dry Equivalent in Grams
Raw Spinach	0.8 to 0.85	0.099 to 0.105
Seven-Minute Spinach	1.3 to 1.4	0.156 to 0.168
Fifteen-Minute Spinach	1.6 to 1.7	0.194 to 0.206
Home Canned Spinach	4.0	0.266
Commercial Canned Spinach	6.0 to 7.0	0.477 to 0.522
Raw String Beans	2.75 to 3.0	0.289 to 0.315
Fifteen-Minute Beans	3.5 to 4.0	0.272 to 0.301
Home Canned Beans	4.25 to 4.75	0.282 to 0.306
Commercial Canned Beans	4.75 to 5.0	0.281 to 0.297
Average gains 5 to 71 per cent.		

*According to Donaldson average normal growth might be considered to be 200 to 300 per cent.

TABLE B18
I. DRY WEIGHT DETERMINATIONS

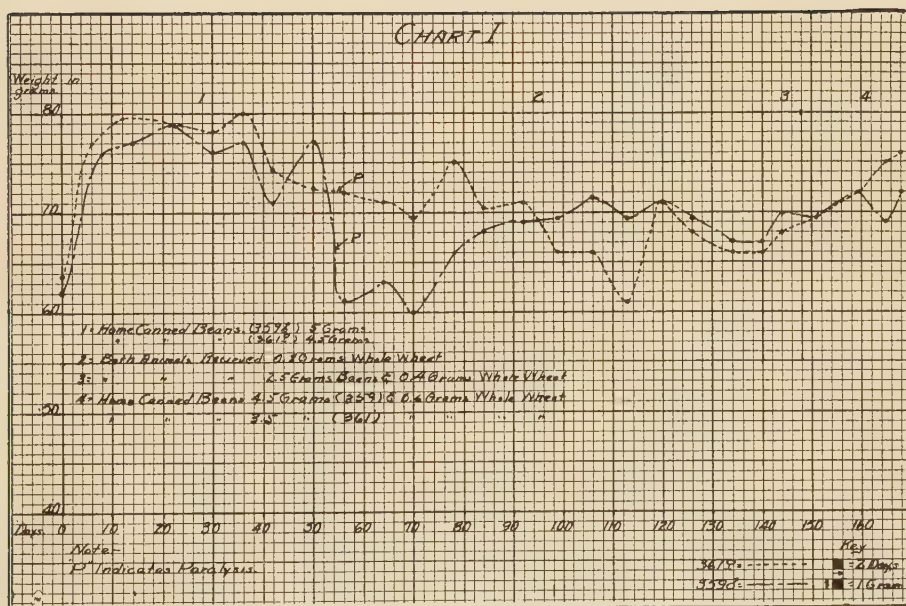
Food	Number Determinations	Number of Samples	Per cent Dry Weight Average
Raw Spinach	6	16	12.498
Seven-Minute Spinach	3	8	11.987
Fifteen-Minute Spinach	4	10	12.122
Home Canned Spinach	3	8	9.562
Commercial Canned Spinach	3	8	7.4023
Raw Beans	4	10	10.4978
Fifteen-Minute Beans	4	9	7.7402
Home Canned Beans	3	8	6.641
Commercial Canned Beans	3	8	55.9487

TABLE B18 (CONTINUED)

II. COMPARISON OF WEIGHTS OF MOIST FOOD WITH DRY WEIGHT EQUIVALENTS.

Food	Grams Moist Food	Dry Equivalent in Grams
Raw Spinach	1.0	0.125
Raw Spinach	0.90	0.112
Raw Spinach	0.88	0.110
Raw Spinach	0.85	0.105
Raw Spinach	0.80	0.099
Seven-Minute Spinach	1.75	0.210
Seven Minute Spinach	1.5	0.177
Seven-Minute Spinach	1.4	0.168
Seven-Minute Spinach	1.2	0.144
Seven-Minute Spinach	1.0	0.120
Fifteen-Minute Spinach	2.0	0.242
Fifteen-Minute Spinach	1.8	0.218
Fifteen-Minute Spinach	1.6	0.194
Home Canned Spinach	5.25	0.502
Home Canned Spinach	5.0	0.479
Home Canned Spinach	4.75	0.454
Home Canned Spinach	4.5	0.431
Home Canned Spinach	4.25	0.406
Home Canned Spinach	4.0	0.382
Libby's Spinach	9.0	0.666
Libby's Spinach	8.0	0.596
Libby's Spinach	7.0	0.522
Libby's Spinach	6.0	0.447
Libby's Spinach	5.0	0.377
Raw Beans	3.75	0.394
Raw Beans	3.25	0.341
Raw Beans	3.0	0.315
Raw Beans	2.75	0.289
Raw Beans	2.5	0.262
Open Kettle Beans	5.0	0.387
Open Kettle Beans	4.75	0.368
Open Kettle Beans	4.5	0.348
Open Kettle Beans	4.25	0.329
Open Kettle Beans	4.0	0.301
Open Kettle Beans	3.75	0.292
Open Kettle Beans	3.5	0.272
Home Canned Beans	5.5	0.365
Home Canned Beans	5.25	0.349
Home Canned Beans	5.0	0.332
Home Canned Beans	4.75	0.315
Home Canned Beans	4.5	0.299
Home Canned Beans	4.25	0.282
Home Canned Beans	4.0	0.266
Snider's Beans	5.5	0.327
Snider's Beans	5.0	0.297
Snider's Beans	4.75	0.281
Snider's Beans	4.5	0.268
Snider's Beans	4.25	0.253
Snider's Beans	4.0	0.238

CHART I



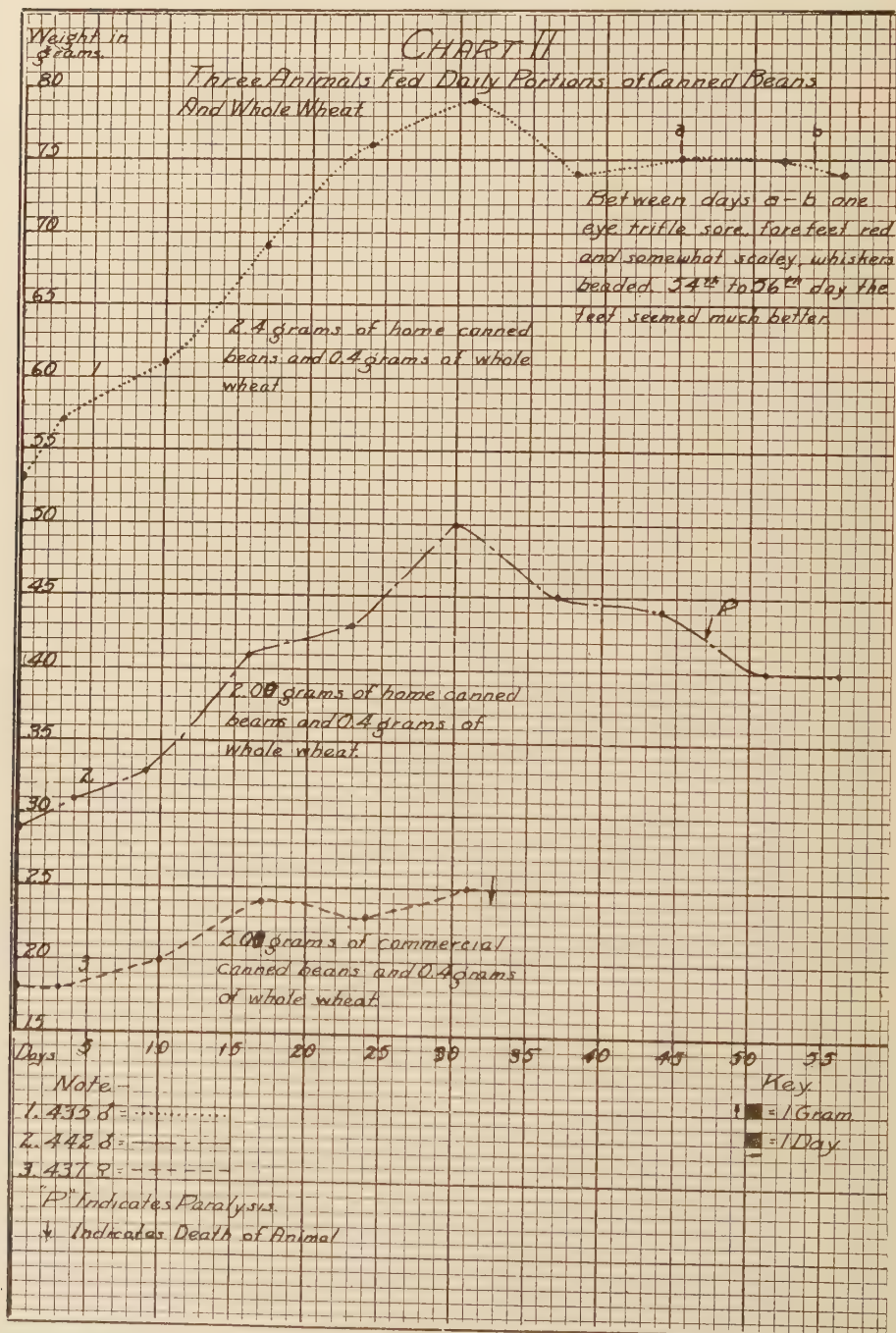
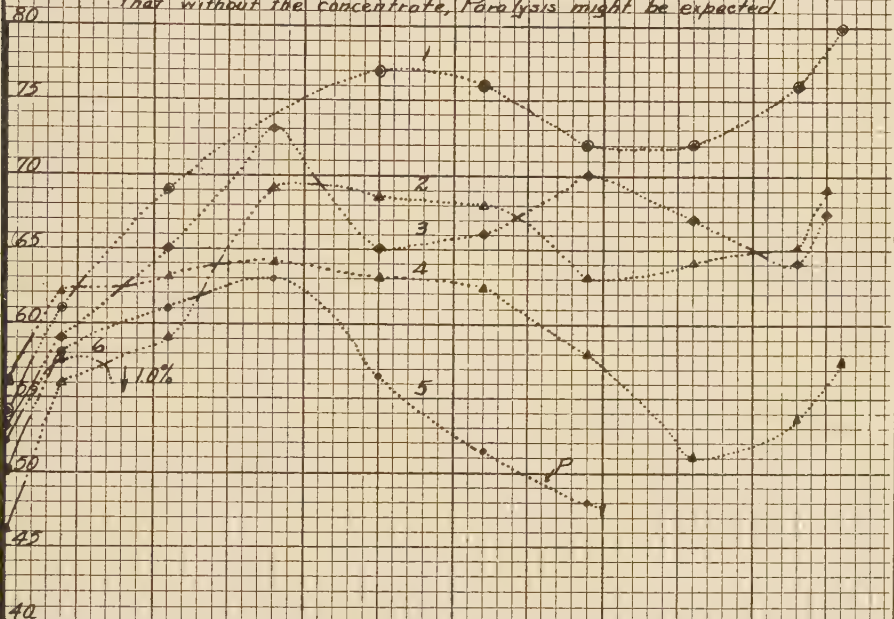


CHART III

Effect of Corn Concentrate in Basal Diet

Raw or canned string beans fed daily, and in portions such that without the concentrate, Paralysis might be expected.



Days 5 10 15 20 25 30 35 40 45 50 55

Note-

- 1-446♂. 3 Grams of raw beans and 2.5% Corn Concentrate.
- 2-450♀. 2.75 Grams of raw beans and 0.5% Corn Concentrate.
- 3-448♀. 3 Grams of raw beans and 1.0% Corn Concentrate.
- 4-445♂. 4.15 Grams of Commercial Canned beans and 2.5% Corn Concentrate.
- 5-449♀. 4.5 Grams of Commercial canned beans and .5% Corn Concentrate.
- 6-447♂. 4.95 Grams of Commercial Canned beans and 1.0% Corn Concentrate.
- 445♂, 446♂ received 2.5% Corn Concentrate in basal diet.
- 447♂, 448♀ received 1.0% Corn Concentrate in basal diet.
- 449♀, 450♀ received 0.5% Corn Concentrate in basal diet.
- 445♂, 447♂ received 4.15 Grams of Commercial Canned beans daily.
- 449♀ received 4.5 Grams of Commercial Canned beans daily.
- 446♂, 448♀ received 3.0 Grams of Raw beans daily.
- 450♀ received 2.75 Grams of Raw beans daily.

"P" Indicates Paralysis.

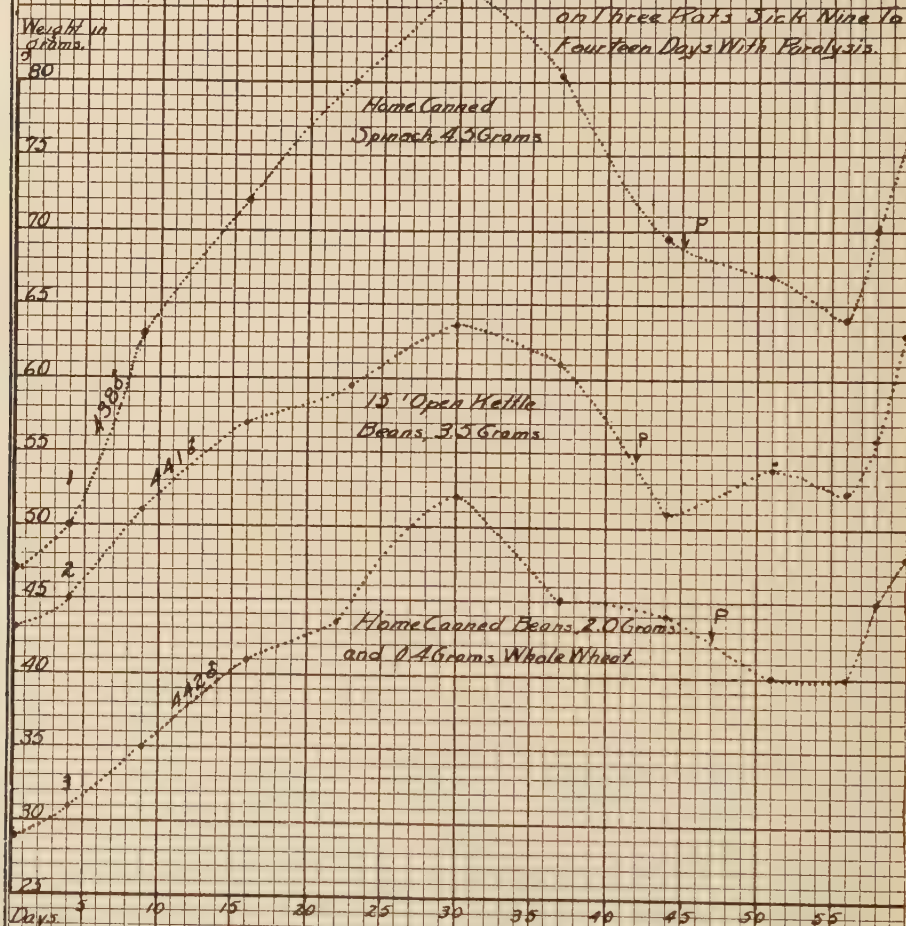
Key.

■ = 1 Gram.

■ = 1 Day.

CHART IV

Effect of Corn Concentrate
on Three Rats Sick Nine to
Fourteen Days With Paralysis.



Note -

1 - Corn Concentrate given as sole source of vitamin B - on 36th day, 25% on 37th day, 25%. On 38th day, 20%. On 39th day, 15%.

2 & 3 - Corn Concentrate added to daily portion of beans at 5% level of basal diet.

"P" Indicates Paralysis.

Key

1 ■ = 1 Gram.

■ = 1 Day

PRELIMINARY REPORT ON VITAMIN A IN SPINACH AND STRING BEANS

In the vitamin A studies the general technique of Sherman and Munsell was used. Casein was purified as described by them²⁵ and the diet in use at present in their laboratory (18 per cent casein, 67 per cent cornstarch, 4 per cent Osborn and Mendel salt mixture, 1 per cent sodium chloride, 10 per cent yeast) was followed in all cases.

Fifty-four animals, 28 to 29 days old, including four negative controls were given this basal diet until they appeared to be depleted of vitamin A. They were then put in separate cages and three times a week they were given small portions of the vitamin food. The basal diet remained the same as in the fore period. They were irradiated with a mercury vapor quartz lamp at a distance of 30 inches from the light, for two minutes each day.

The units for raw spinach and raw string beans given by Munsell were used as the basis of quantities fed. Thirty-six rats were fed graded portions of raw string beans varying from 0.3 to 0.2 gram; of open kettle beans varying from 0.36 to 0.21 gram; of home canned beans, 0.42 to 0.24 gram; of commercial canned, 0.42 to 0.23 gram.

Fourteen animals all told received spinach. Three had 0.016 to 0.019 gram of raw spinach, three 0.0165 to 0.019 of seven-minute cooked spinach, two received 0.0185 and 0.02 gram of fifteen-minute cooked spinach, three 0.018 to 0.025 of home canned, and three 0.018 to 0.025 of commercial canned spinach.

It is at once apparent (see table A1) that there were more irregularities of gain than we have been led to expect from this method. It is possible that some of the animals that showed relatively too large gains had not been entirely depleted of A in the fore period. That is, in each litter there may have been two or three animals that were not depleted at the time when the majority of the litter had shown loss of weight for two or three days.²⁶ It now appears that this may have been a transient loss, and that a steady decline for five or six days would have been a better indication provided that the animal did not become sick. In nearly all cases animals began to be "bleary" eyed before they began to lose in weight.

It will also be noted from table A1 that there was wide variation in the weight of animals at the beginning of the test period, a variation 52 to 118, or 66 grams in the initial weight. The animals that were the largest frequently showed smaller gains than others that received similar amounts of vitamin food. For example animals 349 ♀ and 364 ♂ each received 0.35 gram of vitamin food. The former had an initial weight of 92 grams and gained 43 grams and the latter weighed 62 grams at the beginning of the test period and gained 59 grams.

One group of animals (372 ♂ to 376 ♂) receiving commercial canned beans had eyes that varied from slightly to considerably inflamed. This condition persisted through the test period although they were treated daily with boric acid. The one with the worst eyes showed the least gain in weight (54 grams) and received the largest amount (0.42 gram) daily of the beans. No other animals except negative controls showed this condition of the eyes so markedly, although most of the animals on beans showed it slightly. The fact that the animals were not irradiated during the pre period rules out the possibility of eye injury by light irradiation before the test period actually began. These animals were very plump and sleek-looking. The condition of the eye lids mentioned seemed the only abnormal condition, but autopsy revealed slightly congested lungs in the case of every animal. In only two cases was pus found in the sublingual or submaxillary glands, and then it was found in the

sublingual gland region. These were negative controls (394 ♂ and 409 ♀). Two negative control animals had badly inflamed lids for three weeks, but two showed this condition slightly.

It is evident that we did not check Dr. Munsell's units for either raw spinach 0.018 gram, or for raw string beans 0.28 to 0.3 gram, since three of our animals receiving an average of 0.0177 gram of raw spinach averaged 56 grams increase in weight, and three receiving an average of 0.22 gram of raw beans gained 63 grams. Our units appear to be much smaller than those referred to above but come very close to the unit obtained by Quinn, Burtis, and Milner in 1926.²⁷ These investigators found that 0.09 gram of raw beans used with a series of animals in 1926 contained as much vitamin A as 0.22 gram fed in 1923.

Bulletin 28 gives 6.3 as the average per cent of solids in raw beans, and 7.7 as the per cent of solids in raw spinach. Table B18 shows that our beans contained 10.5 per cent solids and that our spinach contained 12.5 per cent. If there is any relation between per cent of solids in these foods and amount of vitamin A, the fact that one unit appears to be approximately half the Munsell unit and equal to the Quinn and Burtis 1926 unit may be explained. Quinn and Burtis suggest that soil and climatic conditions may affect the vitamin A content.

Although there were the irregularities in gain already referred to, a comparison of gains in weights of animals in the different kinds of beans and spinach shows that there was no loss of vitamin A due to cooking or canning. For example gains in weight of animals 362 ♂ receiving 0.27 gram raw beans, and 368 ♀ and 371 ♀ each receiving 0.35 gram home canned beans, shows that all three obtained the same quantity of vitamin A. This might be expected since 0.27 gram raw and 0.35 gram home canned beans are approximately dry weight equivalents and the relative thermostability of the A vitamin has been demonstrated by Sherman and Quinn.²⁸ Burton³ also found no loss of vitamin A in cooked collards and turnip greens.

This work suggests that it would be helpful if dry weight equivalents of fruits and vegetables should accompany all determinations of vitamin units; also that it might be valuable to know the composition and sources of such foods.

TABLE A1

Serial Number and Sex	Initial Weight In Pre- Period in Grams	Num- ber Days Pre- Period	Vitamin Food	Amount Vitamin Food	Initial Weight in Test Period in Grams	Final Weight in Test Period in Grams	Gain in Grams	Num- ber Days Test Period
344♂	46	30	Raw Beans	0.30	108	149	41	56
355♂	36	35	Raw Beans	0.29	65	65	0	5
347♀	50	30	Raw Beans	0.28	99	153	54	56
362♂	37	35	Raw Beans	0.27	97	147	50	56
369♀	35	35	Raw Beans	0.27	88	121	23	56
365♀	38	35	Raw Beans	0.26	97	131	34	56
386♂	47	23	Raw Beans	0.24	80	175	95	56
389♀	45	23	Raw Beans	0.22	65	119	54	56
392♂	63	28	Raw Beans	0.20	116	154	38	56
348♀	46	30	Open Kettle Beans	0.37	90	135	45	56
353♂	41	35	Open Kettle Beans	0.37	90	154	64	56
349♀	42	30	Open Kettle Beans	0.35	92	135	43	56
			Open Kettle Beans	0.35	62	121	59	56
354♂	40	35	Open Kettle Beans	0.34	79	164	85	56
363♂	36	35	Open Kettle Beans	0.34	95	164	69	56
351♀	37	30	Open Kettle Beans	0.33	79	121	42	56
387♂	44	23	Open Kettle Beans	0.30	70	124	54	56
395♂	29	28	Open Kettle Beans	0.23	56	67	11	18
402♀	55	35	Open Kettle Beans	0.21	93	125	32	56
346♂	35	27	Home Canned Beans	0.42	87	150	63	56
366♀	37	35	Home Canned Beans	0.39	91	91	0	4
350♀	40	30	Home Canned Beans	0.39	86	137	51	56
367♀	37	35	Home Canned Beans	0.37	82	64	-18	6

TABLE A1 (CONTINUED)

Serial Number and Sex	Initial Weight in Pre-Period in Grams	Number Days Pre-Period	Vitamin Food	Amount Vitamin Food	Initial Weight in Test Period in Grams	Final Weight in Test Period in Grams	Gain in Grams	Number of Days Test Period
368 ♀	37	35	Home Canned Beans	0.35	54	106	52	56
371 ♀	35	35	Home Canned Beans	0.35	95	147	52	56
352 ♀	34	30	Home Canned Beans	0.33	84	79	-5	7
356 ♂	27	35	Home Canned Beans	0.33	75	142	67	56
388 ♀	52	23	Home Canned Beans	0.30	82	154	72	56
401 ♀	55	36	Home Canned Beans	0.24	99	141	42	56
372 ♂	51	29	Commercial Canned Beans	0.42	115	166	51	56
373 ♂	47	29	Commercial Canned Beans	0.39	96	179	83	56
375 ♂	43	29	Commercial Canned Beans	0.39	96	170	74	56
374 ♂	46	29	Commercial Canned Beans	0.35	89	148	59	56
376 ♂	33	29	Commercial Canned Beans	0.35	75	169	94	56
385 ♂	55	23	Commercial Canned Beans	0.33	93	171	78	56
393 ♂	52	28	Commercial Canned Beans	0.23	95	144	49	56
403 ♀	51	36	Raw Spinach	0.019	87	153	66	56
396 ♀	49	28	Raw Spinach	0.018	86	112	26	56
411 ♀	43	32	Raw Spinach	0.016	67	153	86	56

TABLE A1 (CONTINUED)

Serial Number and Sex	Initial Weight in Pre-Period in Grams	Number Days Pre-Period	Vitamin Food	Amount Vitamin Food	Initial Weight in Test Period in Grams	Final Weight in Test Period in Grams	Gain in Grams	Number of Days Test Period
398 ♂	56	36	7 Minute Open Kettle Spinach	0.019	118	176	58	56
408 ♀	54	32	7 Minute Open Kettle Spinach	0.018	94	178	84	56
412 ♀	37	32	7 Minute Open Kettle Spinach	0.0165	51	120	69	56
397 ♂	60	36	15 Minute Open Kettle Spinach	0.02	107	146	39	56
410 ♀	43	32	15 Minute Open Kettle Spinach	0.0185	56	137	81	56
399 ♀	57	36	Home Canned Spinach	0.025	104	164	60	56
404 ♂	56	32	Home Canned Spinach	0.020	110	209	99	56
407 ♂	39	32	Home Canned Spinach	0.018	52	47	-5	8
400 ♀	55	36	Commercial Canned Spinach	0.025	102	154	52	56
405 ♂	52	32	Commercial Canned Spinach	0.02	90	165	75	56
406 ♂	43	32	Commercial Canned Spinach	0.018	67	188	121	56
Total Average =		32 days						

TABLE A1 (CONTINUED)

Serial Number and Sex	Initial Weight in Pre-Period in Grams	Number Days Pre-Period	Vitamin Food	Amount Vitamin Food	Initial Weight in Test Period in Grams	Final Weight in Test Period in Grams	Gain in Grams	Number of Days Test Period
345♂	36	29	Negative Controls	67	45	-22	16	45
370♀	35	35		83	82	-1	25	60
394♂	43	28		90	76	-14	41	69
409♀	50	32		70	84	14	39	71
456♀	39	22		57	48	-9	8	29
Averages	41	29		73	51	-6.4	26	55

BIBLIOGRAPHY

1. Kohman, E. F., Eddy, W. H., Vitamins in Canned Foods. V. Peaches. *Ind. and Eng. Chem.*, 18, 302-3, (1926).
2. Kohman, E. F., Eddy, W. H., and Halliday, Nellie, Vitamins in Canned Foods. VI. Strawberries. *Ind. and Eng. Chem.*, 20, 202-4, (1928).
3. Burton, G. W., Quantitative Determination of Vitamins A, B and C in Collards and Turnip Greens. *J. Home Ec.*, 20, 35-42, (1928).
4. Eddy, W. H., Kohman, E. F., and Carlson, Victoria, Vitamins in Canned Foods. III. Canned Spinach. *Ind. and Eng. Chem.*, 17, 69-74, (1925).
5. Goldberger, Joseph, Wheeler, G. A., Lilie, R. D., Rogers, L. M., *Public Health Reports* 41, 297-318, (1926).
6. Hauge, Sigfred M., and Carrick, Carey W., A Differentiation Between the Water-Soluble Growth-Promoting and Antineuritic Substances. *J. Biol. Chem.*, 69, 403-14, (1926).
7. Randoin, Mme. L., and Lecog, M. R., Inégalité de la teneur en vitamines hydro-solubles (B) d'extraits de levure d'origine différente. *Comptes Rendus des l'Acad. des Sc.*, 182, 1408-10, (1926).
8. Salmon, W. D., On the Existence of Two Active Factors in the Vitamin B Complex. *J. Biol. Chem.*, 73, 483-97, (1927).
9. Chick, Harriette, and Roscoe, Margaret Honors, On the Composite Nature of the Water-Soluble Vitamin. *Biochem. J.*, 21, (3), 698-711, (1927).
10. Sherman, H. C., and Axtmeyer, J. H., A Quantitative Study of the Problem of the Multiple Nature of Vitamin B. *J. Biol. Chem.*, 75, 207-12, (1927).
11. Emmett, A. D., and Luros, G. O., Water-Soluble Vitamins. I. Are the Antineuritic and the Growth-Promoting Water-Soluble B Vitamins the Same? *J. Biol. Chem.*, 43, 265-80, (1920).
12. Sherman, H. C., *Chemistry of Food and Nutrition*, Chap. XIV, p. 406.
13. Eddy, Walter H., Hunting the Vitamin. *Amer. J. Pub. Health*, 18, 313-30, (1928).
14. Sherman, H. C., and Spohn, Adela de, A Critical Investigation and an Application of the Rat Growth Methods for the Study of Vitamin B. *J. Amer. Chem. Soc.*, 45, 2719, (1923).

15. Steenbrock, H., Sell, Mariana T., and Nelson, E. M., A Modified Technique in the Use of the Rat for Determination of Vitamin B. *J. Biol. Chem.*, 55, 399-410, (1923).
16. Osborne, Thomas B., and Mendel, LaFayette B., Quantitative Aspects of the Role of Vitamin B in Nutrition. *J. Biol. Chem.*, 54, 739-52, (1922)
17. Osborne, Thomas B., and Mendel, LaFayette B., With the cooperation of Ferry, Edna L., and Wakeman, Alfred J., The Vitamines in Green Foods. *J. Biol. Chem.*, 37, 187-200, (1919).
18. Chamberlain, Weston P., and Vedder, Edward B., Second Contribution to the Etiology of Beriberi. *Phil. J. Sc., Sect. B*, 6, 395-404, (1911).
19. Sherman, H. C., and Grose, M. R., A Quantitative Study of the Destruction of Vitamin B by Heat. *J. Amer. Chem. Soc.*, 45, 2728-38, (1923).
20. Osborne, Thomas B., and Mendel, LaFayette B., with the cooperation of Cannon, Helen C., The Role of Vitamin B in Relation to the Size of Growing Rats. *J. Biol. Chem.*, 63, 233-38, (1925).
21. Sherman, H. C., and MacArthur, E. H., A Quantitative Study of the Determination of Vitamin B. *J. Biol. Chem.*, 74, 107-15, (1927).
22. Eddy, W. H., Kohman, Edward F., and Carlsson, Victoria, Vitamins in Canned Foods. IV. Green Peas. *Ind. and Eng. Chem.*, 18, 85-9, (1926).
23. Sherman, H. C., and Burton, G. W., Effect of Hydrogen Ion Concentration Upon the Rate of Destruction of Vitamin B upon Heating. *J. Biol. Chem.*, 70, 639-45, (1926).
24. Evans, Herbert M., and Burr, George O., A New Differentiation Between the Antineuritic Vitamin B and the Purely Growth Promoting Vitamin B. *J. Biol. Chem.*, 77, 231-40, (1928).
25. Sherman, H. C., and Munsell, H. E., The Quantitative Determination of Vitamin A. *J. Amer. Chem. Soc.*, 47, 1639-46, (1925).
26. Sherman, H. C., and Hessler, M. C., Experiments Upon the Quantitative Differentiation of Vitamins A and D. *J. Biol. Chem.*, 73, 113-20, (1927).
27. Quinn, E. J., Burtis, M. P., and Milner, E. W., Quantitative Studies of Vitamins A, B, and C in Green Plant Tissues other than Leaves. *J. Biol. Chem.*, 72, 557-63, (1927).
28. Sherman, H. C., and Quinn, Edmund, The Relative Thermostability of Vitamin A. Dissertation, Columbia (1925).

ACKNOWLEDGMENTS

This problem was suggested by and was started under the direction of Dr. Hannah Stillman Bradfield, formerly assistant professor in the Home Economics Department. It is a pleasure to acknowledge her help and interest.

The major portion of the work was done under the direction of Dr. Margaret C. Hessler, whose continued interest and helpfulness are gratefully acknowledged.

The author also thanks Dr. Richard Bradfield for the use of the Leeds Northup potentiometer, and help in making the hydrogen ion determinations, and Miss Bertha K. Whipple for supervision of the work during the summer of 1927.

